Remarks

Claims 15-18, 20-23, 25, 27-30, 35-37, 42, and 45-47 are pending. Claims 15-18, 20, 28, 29, and 42 have been amended and new claims 45-47 have been added. The claims are directed to compositions for determining the concentration of a lipoprotein, apolipoprotein, or lipid associated with a lipoprotein in a sample and methods and compositions for determining the ratio of lipoproteins in biological samples.

Restriction Requirement

In the Office Action, the claims were divided into two groups: group I, claims 1-39, 41, and 42, drawn to a composition comprising an antibody which binds a stable conformational independent epitope present on a lipoprotein or apolipoprotein and is uninfluenced by the lipid content which defines the special technical feature, a first method of making the composition and a method of using the composition to determine the concentration of a lipoprotein or apolipoprotein in a sample; and group II, claims 40, 43, and 44, drawn to a second method of using the composition to purify the lipoprotein or apolipoprotein.

Applicants elect Group I, claims 1-39, 41, 42 and newly added claims 45-47. Non-elected claims 40, 43, and 44 are cancelled, without prejudice or disclaimer. Because claims 1-14, and 31-34 are being prosecuted in related applications, U.S.S.N. 08/268,809 and a Continuation application thereof, those claims are also cancelled herein.

Appendix: Pending Claims as Amended

15. (amended) A [The] method [of claim 13] for determining the relative ratio of VLDL to HDL in a biological sample comprising

determining [the amount of VLDL in a sample based on] the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing [soluble] antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3,

[mixing] contacting the [soluble] antibody reactive with Apo C-III with the biological sample to form complexes between the [soluble] antibody and the Apo C-III containing lipoprotein particles,

[adding] contacting the [immobilized] Pan B antibody with [to] the biological sample, and

determining the amount of Apo C-III associated with Apo B, which is the amount of Apo C-III present in VLDL in the sample; and

determining [the amount of HDL in a sample based on] the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$ and $AIbE_2$,

providing [soluble] antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3,

[mixing] contacting the [soluble] antibody reactive with Apo C-III with the biological sample to form complexes between the [soluble] antibody and the Apo C-III containing lipoprotein particles,

[immersing the immobilized] contacting the anti-Apo A-I antibody [into] with the biological sample, [and]

determining the amount of Apo C-III associated with Apo A-I, which is the amount of Apo C-III present in HDL in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample and Apo C-III present in HDL in the sample which is the ratio of VLDL to HDL.

16. (amended) A [The] method [of claim 13] for determining the relative ratio of VLDL to HDL comprising

determining [the amount of VLDL in a sample based on] the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing [a mixture of soluble] antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB_1 which binds to Apo E associated predominantly with VLDL [and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3 which binds to Apo E associated predominantly with HDL],

[adding the mixture of soluble] contacting the antibodies reactive with Apo E associated with VLDL with [to] the biological sample to form complexes between the [soluble] antibodies and Apo E containing particles,

[immersing the immobilized] contacting Pan B antibody [into] with the biological sample, and

determining the amount of Apo E associated with Apo B which is the Apo E present predominantly in VLDL in the sample; and

determining[the amount of HDL in a sample based on] the amount of Apo E present in the HDL in the sample by

providing Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$,

providing [a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB_1, which binds to Apo E predominantly associated with VLDL, and soluble] antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3, which binds to Apo E predominantly associated with HDL,

[adding the mixture of soluble] contacting the antibodies reactive with Apo E to the biological sample to form complexes between the [soluble] antibodies and Apo E containing particles, [and]

contacting Pan B antibody with the biological sample,

determining the amount of Apo E associated with Apo A-I, which is the amount of Apo E present in HDL in the sample, and.

determining the ratio of Apo E present in VLDL in the sample and Apo E present in HDL in the sample which is the ratio of VLDL to HDL.

17. (amended) A [The] method [of claim 13] for determining the relative ratio of LPA-I and [LPA-II] LPA-I:A-II lipoprotein particles in a biological sample comprising

providing anti-Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$,,

providing anti-Apo A-II antibody immunoreactive specifically with Apo A-II having a binding affinity and specificity similar to CdB_5;

[mixing] contacting the [soluble] anti-Apo A-I antibody having a binding affinity and specificity similar to $A1bE_2$ with the sample to form complexes with both LPA-I and [LPA-I:AII] LPA-I:A-II [;]

[immersing the anti-Apo A-I antibody into the biological sample] and determining the quantity of Apo A-I associated with both LPA-I and [LPA-II] LPA-I:A-II lipoprotein particles; and

[immersing] contacting the anti-Apo A-II antibody [into] with the biological sample to form complexes with LPA-I:A-II and determining the quantity of [Apo A-I] Apo A-II associated with the [LPA-I:AII] LPA-I:A-II.

18. (amended) A composition for determining the concentration of a lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein in a biological sample comprising:

[a solid phase material having immobilized thereon] antibody molecules specifically immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and antibody fragments that specifically [binds] bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

20. (amended) The composition of claim 18 wherein the antibodies are [antibody is selected from the group consisting of] monoclonal antibodies[, recombinant antibodies, and antibody fragments].

21. The composition of claim 18 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

22. The composition of claim 18 wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

23. (amended) The composition of claim 18 further comprising [a solution containing molecules of] a second [soluble] antibody immunoreactive with a second distinct epitope of the lipoprotein or apolipoprotein which is immunoreactive with the first antibody [molecules immobilized on the solid phase material].

25. (amended) The composition of claim [19] further comprising at least one internal standard comprising a known amount of a particular lipoprotein, lipoprotein lipid, or apolipoprotein [immobilized on the solid phase material].

27. The composition of claim 18 wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

28. (amended) The composition of claim 18 for determining the relative ratio of VLDL to HDL comprising

[immobilized] Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

[soluble] antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3, and

[immobilized] Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$ and $AIbE_2$[, and

soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA_3].

29. (amended) The composition of claim 18 for determining the relative ratio of VLDL to HDL comprising

[immobilized] Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

[a mixture of soluble] antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB_1 which predominantly binds to Apo E associated with VLDL [and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3 which predominantly binds to Apo E in HDL],

[immobilized] Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to $AIbD_5$, and

[a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB_1 which binds to Apo E predominantly associated with VLDL and soluble] antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD_3 which predominantly binds to Apo E in HDL.

30. The composition of claim 18 for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

[immobilized] Apo-A-I antibody which binds Apo A-I lipoproteins in human plasma having a binding affinity and specificity with Apo $AIbD_5$; and

[immobilized] Apo A-II antibody immunoreactive specifically with Apo A-II having a binding affinity and specificity similar to CdB_5.

35. An antibody molecule specifically immunoreactive with LDL that does not significantly cross-react with other lipoproteins in whole blood, blood plasma or blood serum, wherein the molecule is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof and wherein the antibody specifically binds to a stable, conformation independent epitope which is uninfluenced by the lipid content.

36. The antibody molecule of claim 35 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

37. The antibody molecule of claim 35 wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

42. (amended) A [The] method [of claim 1] for determining the relative ratio of LDL to HDL in a biological sample comprising

adding to the sample antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

adding to the sample antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein.

43. (new claim) A method for determining the relative ratio of first and second lipoproteins in a biological sample, comprising:

determining the amount of first lipoprotein in the sample by

contacting a first antibody immunoreactive with a first apolipoprotein on the first lipoprotein with the sample to form complexes between the first antibody and the first apolipoprotein,

contacting a second antibody immunoreactive with a second apolipoprotein on the first lipoprotein with the sample to form complexes between the second antibody and the first antibody:first lipoprotein complexes,

determining the amount of second apolipoprotein associated with the first apolipoprotein, which is the amount of second apolipoprotein associated with the first lipoprotein;

determining the amount of second lipoprotein in the sample by

contacting a third antibody immunoreactive with a third apolipoprotein on the second lipoprotein with the sample to form complexes between the third antibody and the third apolipoprotein,

contacting a fourth antibody immunoreactive with a fourth apolipoprotein on the second lipoprotein with the sample to form complexes between the fourth antibody and the fourth antibody:second lipoprotein complexes,

determining the amount of fourth apolipoprotein associated with the third apolipoprotein, which is the amount of fourth apolipoprotein associated with the second lipoprotein; and

determining the ratio of first and third apolipoproteins which is the ratio of first and second lipoproteins.

44. (new claim) The method of claim 43, wherein the first apolipoprotein is the same as the third apolipoprotein and at least one of the second or fourth apolipoprotein is specific for the first or second lipoprotein, respectively.

45. (new claim) The method of claim 43, wherein the first antibody is the same as the third antibody.